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L9	I5 and cholesterol	13	L9
L8	L7 and cholesterol	1	L8
L7	sporolactobacillus	55	L7
L6	I5 and (I1 or I2)	2	L6
L5	bacill\$3 near3 coagulan	473	L5
L4	I3 and (bacill\$3 with coagulan)	0	L4
L3	I1 and I2	490	L3
L2	serum near3 (hdl or (high near density near lipoprotein))	669	L2
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FILE 'BIOSIS' ENTERED AT 15:46:12 ON 20 MAY 2002  
L1        514 S (BACILLUS COAGULAN) OR (B COAGULAN)  
L2        59 S SPOROLACTOBACILL##  
L3        118856 S CHOLESTEROL  
L4        4 S L1 AND L3  
L5        0 S L2 AND L3  
L6        0 S L2 AND (LIPOPROTEIN)  
L7        0 S L2 AND SERUM  
L8        0 S L2 AND HDL

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L10      1 S L9 AND CHOLESTEROL

FILE 'CAPLUS' ENTERED AT 15:50:13 ON 20 MAY 2002  
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L12      1 S L10  
L13      0 S L12 NOT L10  
L14      1 S L8 OR L7  
L15      0 S L14 NOT L12  
L16      0 S L6  
L17      692 S L1  
L18      12 S L17 AND L3  
L19      0 S L18 NOT L4

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**From:** Davis, Ruth  
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FUKUSHIMA et al.  
"The effect of a probiotic in faecal and liver lipid classes in rats"  
British Journal of Nutrition, 1995, Vol.73, No.5, p 701-710  
ISSN: 0007-1145

Thanks!

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## The effect of a probiotic on faecal and liver lipid classes in rats

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The effect of a probiotic composed of *Bacillus*, *Lactobacillus*, *Streptococcus*, *Saccharomyces* and *Candida* species (each at  $10^{7-8}$  colony-forming units (cfu)/g rice bran), given at a level of 150 g/kg diet for 6 weeks, on lipid metabolism was examined in the faeces, serum and liver of male rats. Liver weight decreased 35% in the rats fed on a high-fat, high-cholesterol diet containing the probiotic. Total cholesterol concentration in the serum was significantly lower in the probiotic group than in the control group throughout the experimental period in rats fed on the high-fat, high-cholesterol diet, and HDL-cholesterol concentration was significantly higher ( $P < 0.05$ ) in the probiotic group than in the control group which was fed for the 6 week experimental period on a basal diet. The serum VLDL + IDL + LDL cholesterol concentrations in the probiotic groups were reduced compared with those of the corresponding control groups. The probiotic groups fed on the high-fat, high-cholesterol diet and the basal diet had lower hepatic cholesterol concentrations than did the corresponding control groups ( $P < 0.05$ ). Hydroxymethylglutaryl coenzyme A reductase (NADPH) (EC 1.1.1.34) activity in the liver was lower in rats fed on the high-fat, high-cholesterol diet with the probiotic. The neutral and acidic steroid concentrations in faeces were higher in the probiotic group than in the control group fed on the high-fat, high-cholesterol diet. *Escherichia coli* decreased and *Bifidobacterium* and *Eubacterium* increased in the faecal microflora of rats fed on the dietary probiotic. *Lactobacillus* in the probiotic groups was higher than that in the control groups. The present study shows that the probiotic promotes *Bifidobacterium* and *Eubacterium* in the faecal microflora, and reduces cholesterol levels in the serum and liver of rats.

Probiotic: Faecal microflora: Cholesterol: Bile acid: Rat

Since it was proved that atherosclerosis may be reduced by controlling serum cholesterol concentrations (Shiomi *et al.* 1990), numerous active substances with hypocholesterolaemic functions have been investigated. Mann (1977) found that large dietary intakes of yoghurt lowered cholesterol in man. However, cholesterol is a major component of the cell membrane and an essential precursor of steroid hormones and bile acids. These synthetic and metabolic systems regulate cholesterol volume. It is considered that cholesterol synthesis and absorption are inhibited and that the metabolism of cholesterol is promoted by fermented milk (Grunewald, 1982; Hitchins & McDonough, 1989). It is reported that bacteria other than *Lactobacillus* also have a hypocholesterolaemic function (Lee *et al.* 1990). *Saccharomyces cerevisiae* is also effective in treating patients with vitamin B-complex deficiency (Spies, 1953). However, the effects of a probiotic mixture on cholesterol synthesis and metabolism may be determined by symbiotic relationships within the intestinal flora, rather than by the effects of a single bacterial species.

The purpose of the present study was to investigate the effect of a probiotic composed of *Bacillus*, *Lactobacillus*, *Streptococcus*, *Saccharomyces* and *Candida* species isolated from brown forest soil (rich litter and humus) on lipid metabolism in rats.

\* For reprints.

## MATERIALS AND METHODS

*Probiotic*

The composition of the probiotic is shown in Table 1. Each microbe was isolated from brown forest soils gathered from western and northern Japan and incubated at 37° until the log phase (7–14 h) on nutrient broth (Becton Dickinson Co. Ltd, Cockeysville, USA) and a broth medium (Nakano & Fischer, 1977) which contained (/l): K<sub>2</sub>HPO<sub>4</sub> 1·4 g, KH<sub>2</sub>PO<sub>4</sub> 4 g, MgSO<sub>4</sub>·7H<sub>2</sub>O 160 mg, CaCl<sub>2</sub>·6H<sub>2</sub>O 80 mg, MnSO<sub>4</sub>·7H<sub>2</sub>O 8 mg, FeSO<sub>4</sub>·7H<sub>2</sub>O 8 mg, diammonium citrate 4 g, sodium acetate 4 g, meat peptone 20 g, yeast extract 5 g and glucose 30 g. They were further combined and fermented with rice bran for 1 week at 37°. The final proportion of each microbe was adjusted to 10<sup>7–8</sup> colony-forming units (cfu)/g rice bran using pure liquid-cultured microbe.

*Animals and diets*

Male F344 rats (Sato *et al.* 1987) were purchased from Japan CLEA Co. Ltd (Tokyo, Japan). All animals were housed individually in cages on a 12 h light-dark cycle. Temperature and humidity were controlled at 23 ± 1° and 60 ± 5% respectively. All animals were fed on a high-fat, high-cholesterol diet containing 10 g cholesterol/kg for 4 weeks (Table 2) as a preliminary to create hypercholesterolaemic rats. In all trials described below, rats were allowed free access to experimental diets and water, and body weight and feed consumption were recorded every other week. All animal procedures described conformed to the principles in the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1985).

*Expt 1.* The rats (8 weeks old) were divided into two groups of ten animals each. One group was fed on the high-fat, high-cholesterol diet containing 150 g probiotic/kg and a control group was fed on the diet containing 150 g rice bran/kg for 6 weeks.

*Expt 2.* The effect of probiotic was also investigated in hypercholesterolaemic rats fed on a basal diet (Table 2). The rats (8 weeks old) were divided into two groups of ten animals each. One group was fed on the basal diet containing 150 g probiotic/kg and a control group was fed on the diet containing 150 g rice bran/kg for 6 weeks.

*Analytical procedures*

All faeces excreted during one day were collected every other week and the weight was recorded.

Blood samples (2 ml) were collected weekly between 08.00 and 10.00 hours from the jugular veins of fed rats. The samples were taken into tubes without anticoagulant and, after standing at room temperature for 2 h, serum was prepared by centrifugation. At the end of the experimental period of 6 weeks the rats were killed by ether inhalation, and the livers quickly removed, washed with cold saline (9 g NaCl/l), blotted dry on filter paper and weighed before freezing for storage.

*Chemical analysis*

Total cholesterol and HDL-cholesterol concentrations in the serum were determined enzymically using commercially available reagent kits (assay kits for the TDX system, Abbott Lab. Co., Irving, USA).

Total lipids were extracted from faeces and liver by a mixture of chloroform-methanol (2:1, v/v; Folch *et al.* 1957). The fatty acids of phosphatidylcholine (PC) in the liver were methylesterified in HCl-methanol (50 ml/l) for 2 h at 125° (Nakano & Fischer, 1977) and assayed with a Shimadzu 14A gas-liquid chromatograph (Kyoto, Japan). Neutral sterols

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Table 1. *Microbial composition of the probiotic\**

<i>Bacillus subtilis</i>
<i>Bacillus natto</i>
<i>Bacillus megaterium</i>
<i>Lactobacillus acidophilus</i>
<i>Lactobacillus plantarum</i>
<i>Lactobacillus brevis</i>
<i>Lactobacillus casei</i>
<i>Streptococcus faecalis</i>
<i>Streptococcus lactis</i>
<i>Streptococcus thermophilus</i>
<i>Saccharomyces cerevisiae</i>
<i>Candida utilis</i>

\* Each microbe was regulated at  $10^{7-8}$  colony-forming units/g rice bran.

Table 2. *Composition of the semi-purified diets (g/kg)*

Component	High-fat, high-cholesterol diet	Basal diet
Casein	206	250
Sucrose	535	648
Vitamin mixture*	10	12
Mineral mixture†	33	40
Maize oil	—	50
Palm oil	206	—
Cholesterol	10	—

\* AIN-76 vitamin mixture (American Institute of Nutrition, 1977).

† AIN-76 mineral mixture (American Institute of Nutrition, 1977).

in faeces and liver were acetylated (Matsubara *et al.* 1990) and analysed by GLC. Acidic steroids in faeces were measured following the method of Grundy *et al.* (1965).

#### Rat liver enzyme preparation

The liver was homogenized in 2 volumes of cold medium containing 50 mM-KCl, 2 mM-MgCl<sub>2</sub>, 20 mM-Tris-HCl (pH 7.6) and 250 mM-sucrose in a Potter-Elvehjem-type homogenizer. After homogenization with only four strokes the mixture was centrifuged at 1000 g for 10 min, and the supernatant fraction was then centrifuged at 12000 g for 15 min. The resulting pellet was called the mitochondrial (Mt) fraction. The supernatant fraction from this centrifugation was further fractionated by centrifugation at 105000 g for 60 min and the resulting pellet was called the microsomal (Ms) fraction. The Mt and Ms fractions were washed by centrifugation at 12000 g for 15 min and at 105000 g for 60 min in the suspending medium, followed by suspension in 150 mM-KCl (pH 7.6) containing 1 mM-EDTA.

#### Determination of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase (NADPH) (EC 1.1.1.34) activity

The present procedure followed the method of Lippe *et al.* (1985) with some modifications based on Yu-Ito *et al.* (1982).

A 1.5 mg sample of protein was suspended in 200 µl 250 mM-NaCl, 50 mM-potassium phosphate (pH 7.2), 10 mM-EDTA and 10 mM-dithiothreitol. The sample was preincubated for 20 min at 37° and the reaction started with 25 µl 300 mM-glucose-6-phosphate, 25 µl 30 mM-NADP, 1 IU glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and 50 µl 0.14 mM-[3-<sup>14</sup>C]HMG-CoA (0.25 MBq/ml). After 30 min incubation at 37° the reaction was stopped with 0.1 ml 2 M-HCl and the sample left for 30 min at 37° to allow lactonization of mevalonic acid. It was then cooled in ice and centrifuged for 10 min at 3000 g. To the supernatant fraction, 10 µl 0.5 M-mevalonolactone (carrier) and 100 mg Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> were added. The final pH of the solution was 6.5. After double extraction with 2 ml benzene, the extract was spotted on a silica-gel TLC plate and developed in benzene-acetone (1:1, v/v). The silica gel of the mevalonolactone region, detected in iodine vapour, was scraped off, transferred to a scintillation vial containing Bray's cocktail and the radioactivity measured with a scintillation spectrometer.

#### *Growth of bacteria in the faeces*

*E. coli* and *Streptococcus* in the faeces were inoculated and grown for 2 d on deoxycholate agar and streptococcal agar (KF; Becton Dickinson Co. Ltd, Cockeysville, USA) plates respectively at 37°. *Bifidobacterium*, *Eubacterium*, *Lactobacillus*, *Bacteroidaceae*, *Clostridium*, *Veillonella* and *Megasphaera* in the faeces were incubated for 5 d on *Bifidobacterium*-selective (BS) agar medium, *Eubacterium*-selective (ES) agar medium, modified *Lactobacillus*-selective (LBS) agar medium (Becton Dickinson Co. Ltd), Neomycin-Brilliant Green Taurocholate-blood (NBGT) agar medium, Neomycin Nagler (NN) agar medium, modified *Veillonella*-selective (VS) agar medium and modified VS agar medium at 37° by the gaspak method according to the procedure of Mitsuoka *et al.* (1964a, b, 1976).

#### *Statistical analysis*

Data are presented as means and standard deviations (SD). The mean and SD for serum total cholesterol for each time point were calculated and plotted as response curves. The serum total cholesterol responses were expressed as the total area under the curve (AUC) between 0 and 42 d. Student's *t* test was used to compare mean differences between the control group and the experimental group.

### RESULTS

#### *Feed intake, rat growth and liver weight*

The rats initially weighed 123.9 (SD 16.4) g and 119.6 (SD 19.2) g and consumed 15.2 (SD 3.1) g and 16.2 (SD 1.5) g diet/d in Expts 1 and 2 respectively. The rats finally weighed 230.0 (SD 26.2) g and 214.7 (SD 30.5) g at the end of the 6 weeks in Expts 1 and 2 respectively. There were no significant differences in these variables between probiotic and control treatments. The dietary probiotic decreased liver weight significantly in contrast with the dietary rice bran (21.7 (SD 5.3) and 33.5 (SD 1.5) g/kg body weight respectively, *P* < 0.01) in Expt 1. The relative liver weights in Expt 2 were generally comparable (average 16.2 (SD 5.9) and 12.0 (SD 1.2) g/kg body weight for the control and probiotic treatments respectively).

#### *Tissue lipid concentration*

Serum total cholesterol responses are presented in Fig. 1. There were significant differences in the areas under the total cholesterol curves between control and probiotic treatments of rats fed on the high-fat, high-cholesterol diet (Table 3). However, those in rats fed on the basal diet with the probiotic were not significantly different from those in the control group.

Table 3 also illustrates the HDL-cholesterol and VLDL + IDL + LDL-cholesterol concentrations in the serum of rats at the end of the 6-week feeding period. The dietary

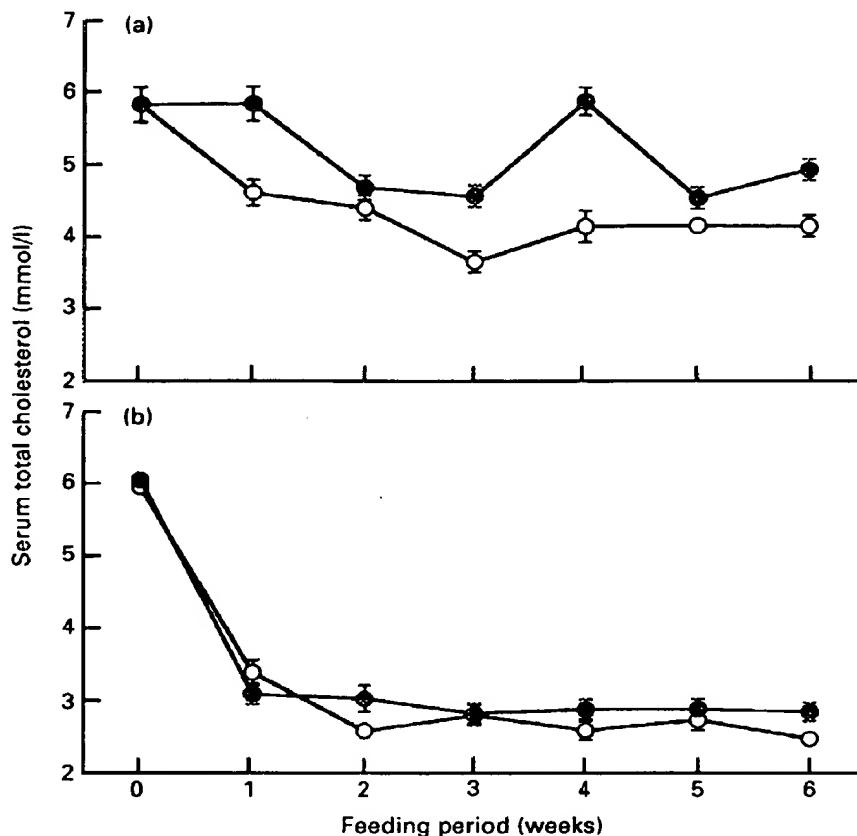


Fig. 1. Serum total cholesterol concentrations in rats fed on (a) a high-fat, high-cholesterol diet or (b) a basal diet, with (○) or without (●) probiotic for 6 weeks. Values are means for ten rats, with standard deviations indicated by vertical bars. For details of diets and procedures see Table 2 and pp. 702-704.

Table 3. Serum total, HDL- and VLDL + IDL + LDL-cholesterol concentrations and liver cholesterol concentration in rats fed on a high-fat, high-cholesterol diet or a basal diet with or without probiotic for 6 weeks†  
(Mean values and standard deviations for ten rats)

Component	High-fat, high-cholesterol diet				Basal diet			
	Control		Probiotic		Control		Probiotic	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<b>Serum (mmol/l)</b>								
Total cholesterol‡	215.0	7.8	180.7**	7.2	132.9	6.8	127.0	6.4
HDL-cholesterol	1.1	0.1	1.3	0.3	1.7	0.1	1.9**	0.1
VLDL + IDL + LDL-cholesterol	3.8	0.3	2.8**	0.2	1.1	0.2	0.5**	0.1
<b>Liver (<math>\mu\text{mol/g}</math> dry liver)</b>								
Cholesterol	105.5	12.9	51.6**	11.0	27.0	5.3	21.9*	2.7

Mean values were significantly different from those of controls: \*  $P < 0.05$ , \*\*  $P < 0.01$ .

† For details of diets and procedures, see Table 2 and pp. 702-704.

‡ Serum total cholesterol values are given as total area under the curve.

Table 4. Fatty acid composition (mol %) of the phosphatidylcholine in the livers of rats fed on a high-fat, high-cholesterol diet or a basal diet, with or without probiotic, for 6 weeks†  
 (Mean values and standard deviations for ten rats)

Fatty acid	High-fat, high-cholesterol diet				Basal diet			
	Control		Probiotic		Control		Probiotic	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
16:0	25.9	3.8	22.5*	2.0	22.6	1.4	22.0	1.3
18:0	22.0	2.8	23.9	1.0	27.7	0.9	27.0	1.6
18:1	18.0	2.7	16.9	1.2	9.4	0.8	9.1	0.6
18:2n-6	14.4	2.3	12.2*	0.8	9.5	1.4	9.2	1.3
20:4n-6	19.4	3.1	24.6**	2.4	30.0	4.1	32.7	1.4
20:4/18:2	1.37	0.22	2.04**	0.35	2.56	0.57	2.80	0.41

Mean values were significantly different from those of controls: \*  $P < 0.05$ , \*\*  $P < 0.01$ .

† For details of diets and procedures, see Table 2 and pp. 702-704.

Table 5. Hydroxymethylglutaryl coenzyme A (HMG CoA) reductase (NADPH) (EC 1.1.1.34) activity (dpm/h per mg protein) in the livers of rats fed on a high-fat, high-cholesterol diet or a basal diet, with or without probiotic, for 6 weeks†  
 (Mean values and standard deviations for ten rats)

Component	High-fat, high-cholesterol diet				Basal diet			
	Control		Probiotic		Control		Probiotic	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Mt fraction	5322	898	4898	724	5612	480	5974	1280
Ms fraction	4658	722	1258**	586	3006	644	2702	282

Mt, mitochondrial; Ms, microsomal.

\*\* Mean value was significantly different from that of the control,  $P < 0.01$ .

† For details of diets and procedures, see Table 2 and pp. 702-704.

probiotic promoted serum HDL-cholesterol concentrations in Expt 2. The VLDL+IDL+LDL-cholesterol concentrations in the probiotic groups were significantly lower than those in the control groups in Expts 1 and 2.

The liver cholesterol concentrations in the probiotic groups decreased significantly compared with those found in the control groups.

#### Fatty acid composition of the liver

Table 4 shows the fatty acid composition of PC in the liver. In Expt 1 the proportion of linoleic acid (18:2n-6) in PC diminished significantly ( $P < 0.05$ ), whereas the proportion of arachidonic acid (20:4n-6) rose in the probiotic group ( $P < 0.05$ ).

When the degree of  $\Delta 6$ -desaturation was estimated as the 20:4/18:2 ratio (Table 4) it was comparable between the control group and the probiotic group in Expts 1 and 2. This ratio increased significantly in the liver PC of rats fed on the high-fat, high-cholesterol diet with the probiotic ( $P < 0.01$ ) but the difference was not significant in rats fed on the basal diet.

**Table 6.** Faecal steroid concentrations ( $\mu\text{mol}/\text{rat per d}$ ) in rats fed on a high-fat, high-cholesterol diet or a basal diet, with or without probiotic, for 6 weeks†  
(Mean values and standard deviations for ten rats)

Component	High-fat, high-cholesterol diet				Basal diet			
	Control		Probiotic		Control		Probiotic	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Coprostanol	10.67	4.49	28.26**	3.42	1.80	0.45	3.32**	1.14
Cholesterol	45.02	15.21	80.09**	12.63	2.94	0.73	3.77*	0.63
CA	0.30	0.30	0.29	0.20	0.05	0.02	0.07*	0.02
DCA	1.19	1.05	1.18	0.67	0.09	0.06	0.12	0.06
CDCA	0.75	0.31	1.43**	0.32	0.06	0.02	0.05	0.02
LCA	1.29	0.61	2.47**	0.82	0.10	0.04	0.10	0.03

CA, cholic acid; DCA, deoxycholic acid; CDCA, chenodeoxycholic acid; LCA, lithocholic acid.

Mean values were significantly different from those of controls: \*  $P < 0.05$ , \*\*  $P < 0.01$  (Student's *t* test).

† For details of diets and procedures, see Table 2 and pp. 702-703.

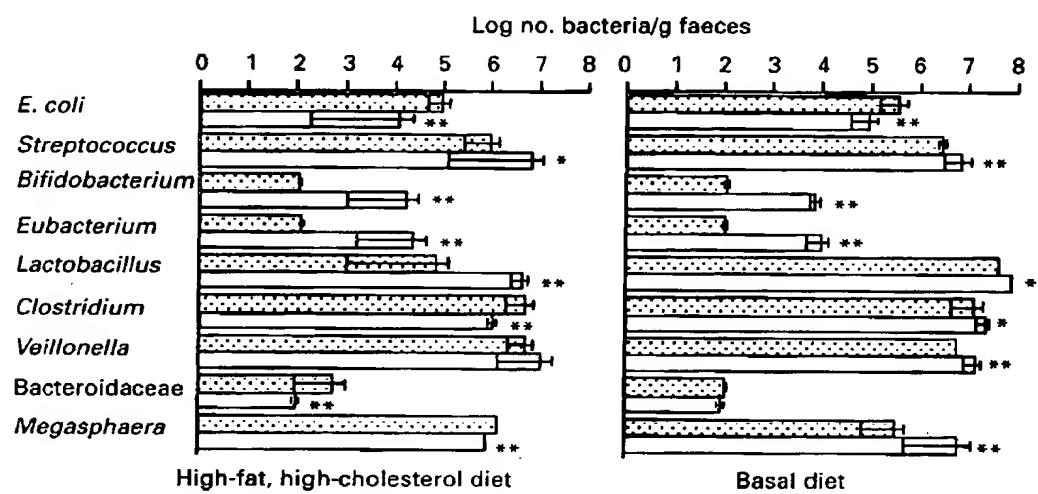


Fig. 2. Composition of the faecal microflora of rats fed on a high-fat, high-cholesterol diet or a basal diet, with (□) or without (▨) probiotic (150 g/kg), for 6 weeks. Values are means for ten rats, with standard deviations indicated by bars. Mean values were significantly different from those of controls: \*  $P < 0.05$ , \*\*  $P < 0.01$  (Student's *t* test). For details of probiotic, diets and procedures, see Tables 1 and 2 and pp. 702-704.

#### HMG-CoA reductase (NADPH) activity in the liver

Table 5 shows the effect of the probiotic on HMG-CoA reductase activity. The radioactivity of this enzyme in the Ms fraction was significantly lower in rats fed on the high-fat, high-cholesterol diet containing the probiotic compared with rats fed on the control diet ( $P < 0.01$ ). That in rats fed on the basal diet containing the probiotic did not show a significant difference compared with that in rats fed on the control diet. There were no significant differences in radioactivity in the Mt fraction among the probiotic and the control groups.

#### Faecal lipid concentration

Table 6 shows the effects of the probiotic on faecal neutral steroid and bile acid concentrations in rats at the end of the experimental period. The dietary probiotic increased

the coprostanol and cholesterol concentrations significantly compared with the corresponding control groups in Expts 1 and 2. The excretion of chenodeoxycholic and lithocholic acids increased significantly in the probiotic group in Expt 1. There was also an increase in the faecal concentration of cholic acid with the basal diet containing the probiotic.

#### *Faecal microflora composition*

Fig. 2 shows the compositions of the faecal microflora of the rats fed on the probiotic for the 6-week experimental period. After 6 weeks, *Bifidobacterium*, *Eubacterium* and *Lactobacillus* increased ( $P < 0.01$ ) in the faeces of rats fed on the probiotic. *E. coli* decreased ( $P < 0.01$ ) in faeces of rats fed on both the high-fat, high-cholesterol diet and the basal diet with the probiotic.

#### DISCUSSION

As shown in Table 1, this probiotic is a microbial mixture. It has been proven that these microbes have individual biological activity (Ozawa & Yokota, 1981; Furushiro *et al.* 1990; Suzuki *et al.* 1991). However, it is possible that the effects of the mixture result from symbiotic relationships in the intestine rather than the effects of individual species. In fact, the probiotic mixture reduced the serum total cholesterol and VLDL + IDL + LDL-cholesterol concentrations in the rats fed on the high-fat, high-cholesterol diet. It also increased the HDL-cholesterol concentration and decreased the VLDL + IDL + LDL-cholesterol concentration in rats fed on a basal diet containing the probiotic. It may be that the synthesis of apolipoprotein B-100, which is the major protein component of circulating VLDL + IDL + LDL (Cardin *et al.* 1984), decreased in the liver and small intestine, or the transfer of cholesterol ester from HDL to VLDL + IDL + LDL (Glomset, 1970) decreased as a result of feeding the probiotic.

The liver weight in rats fed on the high-fat, high-cholesterol diet reduced in the probiotic groups and the hepatic cholesterol concentrations in the probiotic groups decreased significantly in Expts 1 and 2. It was also demonstrated that oral administration of the probiotic mixture decreased HMG-CoA reductase activity in Expt 1. As a general rule, dietary cholesterol accumulates in the liver and suppresses the activity of HMG-CoA reductase, because cholesterol is an inhibitor of this enzyme. It has been reported that the lactone form of compactin, which has been isolated from cultures of *Penicillium citrinum*, is a potent inhibitor of cholesterol synthesis, inhibiting HMG CoA reductase (NADPH) (EC 1.1.1.34), the rate-limiting enzyme in the cholesterol synthetic pathway (Kaneko *et al.* 1978). The data from the probiotic group suggested that this lowering effect was greater than in the control group. From this result it is possible that oral administration of the probiotic regulates the feedback control of the cholesterol synthetic mechanism in the liver which operates during supplementation with cholesterol. Consistent with the change in  $\Delta 6$ -desaturase activity, the linoleate desaturation index, (20:3 + 20:4)/18:2, in rat-liver microsomal PC decreased when cholesterol was fed at more than 5 g/kg diet (Lee *et al.* 1991). The modulation of the fatty acid composition may in turn cause a change in membrane fluidity and, hence, its function. The probiotic may have such a function.

The excretion of chenodeoxycholic acid and lithocholic acid, which cannot be reabsorbed by the intestine, and cholesterol increased in rats fed on the high-fat, high-cholesterol diet. It has been shown that *L. acidophilus* contributes to the elimination of bile acids and cholesterol in the faeces by its binding action and the inhibition of micelle formation (Gilliland *et al.* 1985; Suzuki *et al.* 1991). It is a possibility that this probiotic decreases bile acid absorption and has an inhibitory effect on cholesterol micelle absorption from the intestine. Elevation of the coprostanol concentration was also shown in the faeces. The

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increment of coprostanol concentration may be an index of the activity of intestinal flora (Arjmandi *et al.* 1992). Our data indicated a tendency for this index to increase as a result of feeding the probiotic to rats in Expt 1.

Changes in the levels of *Bifidobacterium* and *Eubacterium* were also observed in the faecal microflora (Fig. 2). Hoffman (1964) showed that a high-fat diet led to a decrease in *Bifidobacterium*. However, in the present experiment, *Bifidobacterium* increased to the  $10^4$  cfu/g level in the faeces of rats fed on the high-fat, high-cholesterol diet with the probiotic compared with only the high-fat, high-cholesterol diet. It may be that the bacteria in this probiotic activated the intestinal flora. It is generally known that *E. coli* can be pathogenic when its numbers increase to more than  $10^4$  cfu/g in the human intestine (Ishibashi & Shimamura, 1993). The reasons for the increases in *Bifidobacterium*, *Eubacterium* and the decrease in *E. coli* were unclear in the present experiment. However, it is a possibility that the lactic acid secreted from *Lactobacillus*, or polysaccharide secreted from each microbe in the probiotic, improved the composition and metabolism of intestinal flora (Fischer *et al.* 1978; Gilliland *et al.* 1978; Nakano & Fischer, 1978). Alternatively, *Lactobacillus* and *Clostridium* may have controlled the number of *E. coli* (Itoh & Freter, 1989), or short-chain fatty acids and antibiotics introduced from *Lactobacillus* in the probiotic may have controlled pathogenic and harmful bacteria (Gilliland & Speck, 1977; Daeschel, 1989). Hitchins & McDonough (1989) investigated whether *Bifidobacterium* from the human intestine was selectively increased by yoghurt bacteria, and the volume of lipids such as cholesterol and triacylglycerol in serum, as well as blood pressure, were improved by an increment of *Bifidobacterium* in patients with hyperlipaemia.

Neither *Candida utilis*, used in food preparation (Peppler, 1970), nor *Monilia albicans*, which causes candidasis infection, were detected in rat faeces. Each organ in rats was also histopathologically normal.

In conclusion, the effect of the probiotic was most clearly seen when it was added to a high-fat, high-cholesterol diet. Its effects were to decrease the serum and liver cholesterol concentrations, increase excretion of neutral and acidic steroids in faeces and inhibit HMG-CoA reductase activity. It is possible that this probiotic improves the balance of intestinal flora and promotes the binding of bile acids and inhibition of micelle formation in the intestine.

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